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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
RAWLINGS, STEPHEN L	

ART UNIT	PAPER NUMBER
1642	15

DATE MAILED: 10/29/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/555,211	STEINLEIN ET AL.	
	Examiner	Art Unit	
	Stephen L. Rawlings, Ph.D.	1642	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 July 2002* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *19-45* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *19-45* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. The amendment filed July 22, 2001 in Paper No. 14 is acknowledged and has been entered. Claims 19, 21, 24, 26-28, 30, 32, 36, 39, and 41-45 have been amended.

2. Claims 19-45 are pending in the application and are currently under prosecution.

Grounds of Objection or Rejection Withdrawn

3. Unless specifically reiterated herein, the grounds of objection or rejection that were set forth in the previous Office action mailed February 26, 2002 (Paper No. 12) are withdrawn.

Grounds of Rejection Maintained and Reply to Applicants' Remarks

Claim Rejections - 35 USC § 112

4. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 45 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Applicants have traversed the grounds of rejection of claim 45 set forth in the previous Office action mailed February 26, 2002, disagreeing with the Examiner and noting that the specification ~~teaches~~ discloses that the claimed method is capable of measuring the influence of gene expression within 24 to 48 hours so that it is possible to analyze and isolate cells while they are still alive.

In reply to Applicants' remarks, while it would be possible to measure the influence of the expression of a cDNA molecule upon the cell's survival within 24 to 48 hours, it is not clear that it would be possible to isolate those cells that express a cDNA molecule that encodes a protein, for example, that induces the onset of apoptosis in the cell, because, as asserted in the previous Office action, once apoptosis has ensued within a cell, the cell and its DNA contents are rapidly destroyed. In the absence of exemplification, the skilled artisan would not accept the assertion that the claimed invention can be practiced with a reasonable expectation of success without the need to first perform additional, undue experimentation. Accordingly, the specification fails to provide an enabling disclosure of the invention that meets the requirements set forth under 35 USC § 112, first paragraph. Therefore, in view of the preponderance of evidence, the state of the art, and level of unpredictability associated with the art, Applicants' arguments have been carefully considered but have not been found persuasive.

6. Claims 19-40, 44, and 45 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

(a) Claims 19-40 are vague and indefinite because claims 19, 26, and 32 recite the phrase "comparing the values" and/or the phrase "comparing the calculated proportion of apoptotic cells". Recitation of the phrase renders the claim vague and indefinite because it cannot be ascertained how the claim requires the values to be compared. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Although Applicants have submitted that the amendment to the claims has obviated this ground of rejection, the issue has not been fully resolved by the amendment. Although the present claims clearly recite the means by which the proportion of transfected cells in the population and the proportion of apoptotic cells in the population are to be determined, the present claims still do not recite how the values

of these proportions are to be compared to determine the proportion of the transfected cells that are apoptotic.

(b) Claims 19-25 are vague and indefinite because claim 19 recites the phrase "determining the proportion of apoptotic cells in the transfected population" in line 16. Recitation of the phrase renders the claim vague and indefinite because it cannot be ascertained how the claim requires the proportion of apoptotic cells in the transfected population is to be determined. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

As noted above, the amendment to the claims has not fully resolved this issue.

(c) Claim 44 is indefinite because the claim recites the term "70% ethanol". Recitation of the terms renders the claims indefinite because it cannot be determined if the concentrations are expressed at unit mass/unit volume or unit volume/unit volume. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Applicants have traversed this ground of rejection arguing that it would be obvious to one skilled in the art that ethanol is prepared unit volume of ethanol/unit volume of solvent. Nevertheless, a solution of 70% ethanol can be prepared by mixing 70 grams of ethanol into 100 milliliters of solvent. 35 USC § 112, second paragraph requires the claims to particularly point out and distinctly claim the subject matter that Applicants regard as the invention, and as it would be prudent to amend the claims as necessary to meet said requirements, Applicants are advised to do so.

(d) Claim 45 is vague and indefinite because the claim recites the phrase "cells which differ from an apoptosis background which is to be determined". Recitation of the phrase renders the claim vague and indefinite because: (a) the term "apoptosis background" is not defined by the claim and it cannot be determined to what measurement the claim refers, (b) it cannot be determined when or how the claim requires the "apoptosis background" to be measured, and (c) it cannot be determined how and to what extent the claim requires the cells to differ from an "apoptosis background", so it cannot be determined which cells the claim requires to be isolated.

Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Applicants have traversed this ground of rejection, submitting that the term "apoptotic background" would be understood by the artisan of ordinary skill, but define the term as "an apoptotic phenotype which, depending on the problem to be solved, is predefined in each individual experiment" (page 30, paragraph 2). Furthermore, Applicants have referenced a disclosure that states, "[f]or this purpose the method is modified by using FACS sorting to isolate single cells which deviate from an apoptosis background which is to be determined". Of course, this disclosure begs the question that if the apoptosis background is to be determined, how might one determine whether single cells deviate from the background before it is determined. Nevertheless, for those reasons noted in the previous Office action mailed February 26, 2002 and reiterated above, the recitation of the phrase renders the claims vague and indefinite. In addition, regarding the disclosure to which Applicants have referred, if cells that have not been transfected with the plasmid containing the DNA sequence of interest are undergoing apoptosis, the number of which represents the value of the "apoptotic background", then how can the practitioner of the claimed method distinguish cells undergoing spontaneous apoptosis, i.e., the background cells, from those cells that have been transfected with a plasmid containing a DNA sequence of interest so that the latter can be isolated to identify the gene that modulates apoptosis?

Claim Rejections - 35 USC § 102

7. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in-

(1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effect under this subsection of a national application published under section 122(b) only if the international application designating the United States was published under Article 21(2)(a) of such treaty in the English language; or

(2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that a patent shall not be deemed filed in the United States for the purposes of this subsection based on the filing of an international application filed under the treaty defined in section 351(a).

8. Claims 19, 21, 22, 23, 25, and 26 are rejected under 35 U.S.C. 102(a) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Keane, et al (*Proc. Annu. Meet. Am. Assoc. Cancer Res.* **38**: A1148, 1997, Abstract No. 1148).

Applicants have traversed this ground of rejection arguing that the prior art is not anticipatory of the claimed invention, because the present claims require the simultaneous measurements of the proportion of the harvested cells containing fluorescent marker protein and the and the proportion of the harvested cells containing a DNA content of less than 2N.

In reply to Applicants' remarks, although Keane, et al do not explicitly teach that the measurements of the proportion of the harvested cells that are transfected, i.e., the cells expressing the fluorescent marker protein, and the proportion of the harvested cells undergoing apoptosis, i.e., the cells that have hypodiploid DNA content, must be made simultaneously or carried out using separate aliquots of the same cell population at the same time, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have done so, and there is no apparent reason that one would not have been compelled to do so, provided the resources were not limiting. The recitation of the limitation requiring the measurements to be made simultaneously, therefore, does not patentably distinguish the method of the prior art from that of the claims.

In addition, Applicants have asserted that as Keane, et al does not explicitly disclose that the proportion of cells undergoing apoptosis was measured using propidium iodide (PI) *after fixation of the cells*, Keane, et al fail teach every element of

the claims. Furthermore, Applicants have stated, "even assuming, *arguendo*, that PI was used to detect apoptosis, [...] a fixing step is not necessary for this method" (page 35, paragraph 1). In support of the contention that it is not necessary to fix the cells so that the DNA content of the cells can be measured, Applicants have referred to "Vermont Cancer Center Flow Cytometry Protocol, pages 2-3. Accordingly, Applicants have submitted, "neither the PI staining step for detecting apoptosis nor the fixing and permeabilizing step are 'necessarily present' in the Keane *et al.* methods" (page 35, paragraph 1). Moreover, Applicants have argued that it would not have been obvious to use PI to stain the DNA of cells to determine which cells are undergoing apoptosis or to fix and permeabilize the cells so that measurements of the numbers of apoptotic cells and transfected cells could be made simultaneously, because there would have been no motivation to do so and one would not have had a reasonable expectation of success in employing such a method. To support this argument, Applicants have noted that Kalejta, et al (*Cytometry* **29**: 286-291, 1997) teach the need for improved methods, since the commonly employed methods resulted in inefficient PI staining and poor quality DNA histograms.

Firstly, contrary to Applicants' statements, Keane, et al discloses, "apoptosis was assessed using propidium iodide (PI)". Secondly, although pages 2 and 3 of the "Vermont Cancer Center Flow Cytometry Protocol" have not been made available to the Examiner, it is known that propidium iodide can and has been used to measure the DNA content of unfixed cells. Perhaps Applicant correctly presumes that Keane, et al did not first fix and permeabilize the cells in determining the DNA content of the transfected cells, but considering the state of the art at the time the invention was made, it would have been obvious to do so. Although methods were known and had been used in which the DNA content of a cell could be determined using PI, and without need to fix the cells, methods were also known and had been used in which the cells are fixed and permeabilized before staining. As the application of these latter methods had demonstrated success, contrary to Applicants' assertion, one of ordinary skill in the art would have had a reasonable expectation of successfully using the claimed method.

In addition, it is noted that Applicants have cited Kalejta, et al to support their assertion that it would not have been obvious to first fix and permeabilize the cells in determining the DNA content of the transfected cells using PI. However, as Applicants have remarked in traversing other grounds of rejection, the publication by Kalejta, et al is not prior art, and even if the disclosure by Kalejta, et al would have dissuaded one of ordinary skill in the art from deriving the presently claimed invention, it was not publicly available to have done so.

Nevertheless, Applicants' have speculated that the methods of Kalejta, et al have remedied problems encountered when the cells are permeabilized using ethanol. However, the disclosure of Kalejta, et al to which Applicants have referred states, 'the use of paraformaldehyde-based fixatives allows GFP to be maintained in cells and retain its fluorescence even after ethanol permeabilization". Additionally, this disclosure states, "[f]or PI to enter cells efficiently and to stain DNA quantitatively, the cell must be first permeabilized". Therefore, absent knowledge of the "remedy" disclosed by Kalejta, et al, but in view of their teachings, it seems that it would have been obvious to one of ordinary skill in the art at the time the invention was made to have fixed and permeabilized the cells before determining the DNA content of the cells using PI. Moreover, as one objective to practicing the claimed method is to make a determination of the proportion of cells undergoing apoptosis, it would have been considered prudent to fix the cells, since otherwise, following cessation of the culturing, the cells undergoing apoptosis would self-destruct and cells that had not initiated apoptosis might do so as a result of either the expression of the DNA sequence of interest or some other non-specific cause, such as mechanical agitation or stress, which might tend to skew the results of the analysis.

Therefore, in reply to Applicants argument that it would not have been obvious to use PI to stain the DNA of cells to determine which cells are undergoing apoptosis or to fix and permeabilize the cells so that measurements of the numbers of apoptotic cells and transfected cells could be made simultaneously, contrary to their assertions, there would have been no motivation *not to have* derived the presently claimed invention, as one *would have had* a reasonable expectation of success in employing such a method.

Finally, Keane, et al disclose:

> Transfected cells were then selected by co-transfection with green fluorescent protein. The percent of apoptotic cells in **selected** DEP-1 transfected cells was [measured] (emphasis added).

Therefore, although it is not explicitly stated that the proportions of apoptotic cells and transfected cells were simultaneously measured *using the same flow cytometer*, any conjecture that it was not done so is not supported by their disclosure, because the term "selected" could have been used to indicate that during the analysis of the acquired data, the population of cells that expressed the fluorescent marker protein was selected, or *gated-on* using suitable computer software to enable the determination of the proportion of the transfected cells that were undergoing apoptosis at the time the cells were fixed. Alternatively, the population of transfected cells could have been electronically *gated-on* during the acquisition of the data using a fluorescence-activated cell sorter, in which case virtually simultaneous measurements of the different proportions would have been made. Even so, the claims do not presently recite a limitation requiring that the measurements of the proportions of apoptotic cells and transfected cells be made simultaneously *using the same apparatus*. Therefore, the claims encompass methods comprising steps in which the measurements are made simultaneously using different instrumentation, and again there is no reason that it would not have been obvious to one of ordinary skill in the art to have done so provided the necessary facilities and resources were available.

Accordingly, Applicants' arguments have been fully considered, but not found persuasive.

Claim Rejections - 35 USC § 103

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said

subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

10. Claims 19-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Keane, et al (*Proc. Annu. Meet. Am. Assoc. Cancer Res.* **38**: A1148, 1997, Abstract No. 1148) in view of Douglas, et al (*Journal of Immunological Methods* **188**: 219-228, 1995), Anderson, et al (*Proceedings of the National Academy of Science USA* **93**: 8508-8511, 1996), and Baker, et al (*Nucleic Acids Research* **25**: 1950-1956, 1997) and in further view of Keane, et al (*Proc. Annu. Meet. Am. Assoc. Cancer Res.* **37**: A299, 1996; Database CANCERLIT, Accession No. 96647249) or in still further view of Sell, et al (*Cancer Research* **55**: 303-306, 1995) and Prager, et al (*Journal of Clinical Investigation* **90**: 2117-2122, 1992) or Chow, et al (*Development* **121**: 4383-4393, 1995) or Strawn, et al (*Journal of Biological Chemistry* **269**: 21215-21222, 1994) or Trent, et al (*EMBO Journal* **15**: 4497-4505, 1996), as evidenced by the teachings of Chu, et al (*Cytometry* **36**: 333-339, 1999).

Applicants have traversed this ground of rejection stating reasons that they believe each of the cited references fail to teach or suggest every element of the claims. Furthermore, Applicants have asserted that without benefit of the instant application's disclosure, the invention would not have been obvious because one would not have had believed the invention could be used successfully.

In reply to Applicants' arguments, Applicants are reminded that one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Furthermore, in response to Applicants' argument that there is no suggestion to combine the references, the Examiner recognizes that obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988) and *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992). In this case,

several reasons that one would have been motivated to derive the invention after combining the teachings of the cited references were set forth in the previous Office action.

Furthermore, several of the cited references teach the simultaneous, but independent measurement of two or more variables using multiparameter flow cytometry. Although Keane, et al do not explicitly teach that the proportions of apoptotic cells and transfected cells in a population of cells can be measured simultaneously using the same instrument, the claims do not recite such a limitation. Given the resources, it would have been obvious to one of ordinary skill in the art to simultaneously measure both parameters simultaneously using different instrumentation. Nonetheless, as multiparameter flow cytometry was obviously conventional at the time the invention was made, because GFP, for example, has excitation and emission properties that differ from those of propidium iodide, for example, one would have had a reasonable expectation of success in practicing the invention without having need of the benefit of Applicants' disclosure.

In addition, Applicants have asserted that citing Chu, et al was improper since it is not prior art. However, as noted in the previous Office action and reiterated herein, Chu, et al was cited as evidence of the likelihood of success in practicing the claimed invention without benefit of Applicants' disclosure.

New Grounds of Claim Rejections

Claim Rejections – 35 USC § 112

11. Claims 19-40 and 45 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods comprising the step of co-expressing in the population of cells a fluorescent marker protein, provided said step is achieved either by co-transfecting the population of cells with a plasmid containing the DNA sequence of interest and DNA encoding the fluorescent marker protein or by transfecting the population of cells with a plasmid containing both the DNA sequence of interest and DNA encoding the fluorescent marker protein, does not reasonably provide enablement for methods comprising the step of co-expressing in the population of cells

a fluorescent marker protein, wherein said population of cells stably expresses the fluorescent marker protein. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The claims are drawn to methods that comprise identifying and/or determining the proportion of cells that are undergoing apoptosis in a population of cells transiently transfected with a plasmid containing a DNA sequence of interest. The claims recite a step in which a DNA encoding a fluorescent marker protein is co-expressed with the DNA sequence of interest. The claims encompass methods in which a population of cells is co-transfected with a plasmid containing the DNA sequence of interest and another plasmid containing a DNA sequence encoding a fluorescent marker protein. In addition, the claims encompass methods in which the population of cells is transfected with a plasmid containing both the DNA sequence of interest and the DNA sequence encoding the fluorescent marker protein. In either case, measuring the proportion of cells that express the fluorescent marker protein would enable a determination of the proportion of transfected cells in the population of cells. However, the claims also encompass methods in which the population of cells that constitutively express the fluorescent marker protein is transfected with the plasmid containing the DNA sequence of interest. In this case, one could not determine the proportion of transfected cells in the population of cells by measuring the proportion of cells expressing the fluorescent marker protein, because all of the cells in the population express the protein, regardless of whether the cells have been transfected with the plasmid containing the DNA sequence of interest. Therefore, it would not be possible use the claimed invention to identify or determine the proportion of cells that are undergoing apoptosis in a population of cells that express the fluorescent marker protein.

12. Claims 19-45 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 19-40 and 45 recite the limitation "simultaneously". However, it does not appear that there is proper and sufficient antecedent basis in the specification for recitation of this limitation in the claims.

Claims 19-40 and 45 recite the limitation "co-expressing". It is noted that the recitation of the limitation "co-expressing" broadens the scope of the original claims, as the original claims did not encompass a method in which the population of cells that is transfected stably expresses the DNA encoding the fluorescent protein. Consequently, there does not appear that there is proper and sufficient antecedent basis in the specification for recitation of this limitation in the claims.

Claims 41-44 recite the phrase "the simultaneous measurement of the fluorescence of marker protein and DNA content". Again, it does not appear that there is proper and sufficient antecedent basis in the specification for recitation of this phrase in the claims.

Therefore, the recitations of the limitation and phrase in the claims appear to introduce new matter, thereby violating the written description requirement set forth under 35 USC §112, first paragraph. These matters might be resolved if in reply to this Office action, Applicants point to specific disclosures in the specification that are believed to provide the necessary support for the recitation of the limitation and phrase.

13. Claims 27, 33, 39, and 41-44 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

(a) Claims 27, 33, and 39 are vague and indefinite because claims 27 and 33 recite the term "signal transduction molecule of a receptor". Recitation of the term renders the claims vague and indefinite because the term is not defined by the claim, nor is the term explicitly defined in the specification. While the specification discloses example of molecules that are considered to be signal transduction, or transmission molecules of a receptor, because the term is not defined, it is still not apparent which molecules are encompassed by the claims, and which are not. Accordingly, one of

ordinary skill in the art would not be reasonably apprised of the metes and bounds of the invention.

(b) Claims 41-44 are indefinite because claim 41 and 42 recite the term "suitable". Recitation of the term renders the claims indefinite because "suitable" is a relative term, which is not defined by the claim. Because the specification does not provide a standard for ascertaining the requisite degree of suitability, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the invention. Amending claim 41 to delete "suitable" can obviate this ground of rejection.

It is noted that the previous Office action contained a typographical error, as the paragraph in which this ground of rejection was set forth suggested, "[a]mending claim 41 to delete "suitable" can obviate this rejection insofar as the rejection pertains to claim 41", which should have read, "[a]mending claim 41 to delete "sufficient" can obviate this rejection [...]". Perhaps this error caused the Applicants some confusion, and the Examiner apologizes for any inconvenience or confusion the error may have caused.

Claim Rejections - 35 USC § 103

14. Claims 19, 21, 22, 23, 25, and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Patent No. 5,976,853-A in view of Endersen, et al (*Cytometry* **20**: 162-171, 1995), Douglas, et al (*Journal of Immunological Methods* **188**: 219-228, 1995), Anderson, et al (*Proceedings of the National Academy of Science of the USA* **93**: 8508-8511, 1996), and Lybarger, et al (*Cytometry* **25**: 211-220, 1996).

US Patent No. 5,976,853-A ('853) teaches that which is set forth in the previous Office action, which was presented as the basis of the rejection of the claims under 35 USC § 102. However, as Applicants have noted, '853 teaches that the measurements of the proportions of apoptotic cells and transfected cells were not made simultaneously, which does not necessarily show that it would not have been obvious to have done so.

Endersen, et al, Douglas, et al, and Lybarger, et al teach methods for the simultaneous or coordinate measurement of two variables using multiparameter flow Cytometry. Endersen, et al and Douglas, et al demonstrate that measurements of two

variables can be made provided that both variables are enumerated by measuring the presence of molecules having different excitation and emission characteristics, such as fluorescein isothiocyanate (FITC) or green fluorescent protein (GFP), phycoerythrin (PE) or propidium iodide (PI), and allophycocyanin (APC). Anderson, et al teach engineered GFP molecules having different excitation and emission properties that can measured simultaneously using the same flow cytometer. Additionally, Enderson, et al teach that the disclosed methodology provides the opportunity to estimate the cell cycle distributions of both the apoptotic and non-apoptotic cell populations, which is especially valuable in assessing the affects of drugs, such as etoposide that cause apoptosis in association with perturbing the cell cycle.

In view of the prior art, it would have been *prima facie* obvious to one of ordinary art at the time the invention was made to have modified the protocol of '853 so that the proportions of apoptotic cells and transfected cells in a population of cells could be determined simultaneously using multiparameter flow cytometry. One of ordinary skill in the art at the time the invention was made would have been motivated to have done so, because Enderson, et al, teach that such methodology provides the opportunity to estimate the cell cycle distributions of both the apoptotic and non-apoptotic cell populations, which is especially valuable in assessing the affects of drugs, such as etoposide, since one could assess the affect of a drug on both populations of cells simultaneously to compare and contrast those effects and correlate the effects with the presence and absence of the plasmid containing the DNA sequence of interest.

Conclusion

15. No claims are allowed.

16. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

17. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephen L. Rawlings, Ph.D. whose telephone number is (703) 305-3008. The examiner can normally be reached on Monday-Thursday, alternate Fridays, 8:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony C. Caputa, Ph.D. can be reached on (703) 308-3995. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Stephen L. Rawlings, Ph.D.
Examiner
Art Unit 1642

ANTHONY C. CAPUTA
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

slr
October 20, 2002